Carcass quality, nutrient retention and Caeca microbial population of broiler chicks administered Rolfe (*Daniellia oliveri*) leaf extract as an antibiotic alternative

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Article History
Received: 24 April 2020
Accepted: 05 June 2020
Published: June 2020

Citation
Olafadehan OA. Oluwafemi RA, Alagbe JO. Carcass quality, nutrient retention and Caeca microbial population of broiler chicks administered Rolfe (*Daniellia oliveri*) leaf extract as an antibiotic alternative. *Drug Discovery*, 2020, 14(33), 146-154

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ISSN 2278–540X; EISSN 2278–5396

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ABSTRACT

The study was designed to evaluate the carcass quality, nutrient retention and caeca microbial population of broiler chicks administered *Daniellia oliveri* leaf extract (DOE) as an antibiotic alternative. A total of 250 – one-day-old broiler chicks (Ross 308) were allocated to five treatments in a completely randomized design and each treatment group was further sub-divided into five replicates containing ten (10) birds each. Treatment 1 (Control) was given 1.20 g/ liter of Neomycin in water, while treatments 2, 3, 4 and 5 were given DOE at 10 ml, 20 ml, 30 ml and 40 ml/liter of water respectively. Clean feed and water were supplied ad libitum throughout the experiment which lasted for 56 days. Data collected were used to determine the carcass quality, nutrient retention and caeca microbial population. Highest dressing percentage was recorded for birds in T5 (70.0%) followed by T4 (69.08 %), T3 (67.18 %), T2 (67.44 %) and T1 (65.0 %) respectively ($P<0.05$). Weights of liver, kidney, spleen, gizzard, heart, intestine and other primal cut parts were significantly influenced ($P<0.05$). Nutrient retained for dry matter, crude protein, crude fibre, ether extract and nitrogen free extract were significantly ($P<0.05$) different among the treatments. Feeding DOE at 40 ml/litre significantly ($P<0.05$) decreased the count of *E.coli* and other pathogenic organisms, lactobacillus count was significantly ($P<0.05$) highest for all treatments fed DOE compared to the control. It was concluded that feeding DOE up to 40 ml/litre could aid in the proliferation of Lactobacilli and does not have any deleterious effect on the performance, carcass quality of broiler chicks.

Keywords: *Daniellia oliveri*; nutrient retention; carcass quality; broiler chicks.

1. INTRODUCTION

The indiscriminate use of antibiotics has made the European Union in 2006 to place a ban on the use of antibiotic growth promoters (AGPs) due to antimicrobial resistance and their residual deposit in animal products leading to health challenges. The use of medicinal plants has now been viewed as one of the suitable organic or botanical alternative to antibiotics, because they are cheap, safe and efficient. WHO (2006), reported that there are over 30,000 species of plants and only a very few percentage has been given consideration in research. Among the potential under-explored plant is *Daniellia oliveri*.

Daniellia oliveri (Rolfe) is a leguminous plant belonging to the family Caesalpiniacea. It is found growing in many parts of Nigeria and widely distributed in Africa, Amazon region, Asia and many parts of South America (Osuntokun *et al*., 2016). Different parts of the plants have been useful medicinally, for instance, the leaves have been used to treat gastro-intestinal parasites (Djoueche *et al*., 2011), tooth ache problems (Adubiaro *et al*., 2016). The roots are also used treat fever and sexually related problems (Osuntokun *et al*., 2016). The plant is rich in phytochemicals or bioactive chemicals such as alkaloids, flavonoids, saponins, phenols, oxalate, tannins and oleoresins (Gilbert, 2000). These chemicals confer the plant the ability to perform multiple biological activities: antibacterial (Akujobi *et al*., 2004), antifungal (Edeoga *et al*., 2005), anti-inflammatory (Astal *et al*., 2005), antiviral activity (Vats et al., 2011), anti-helminthic (Cowan, 2005) and antioxidant (Facey *et al*., 1999).

Previous studies have revealed *Daniellia oliveri* exhibit a wide range of pathogenic organisms (Wong et al., 2008). Extracts from clove, tea, garlic, cinnamon and others have potential anti−inflammatory activities and suppress the production of TNF-α, IL-1β and NO from LPS-induced mouse macrophages (Lee et al., 2005). A synergistic combination of various bioactive chemicals causes negative consequences on enteric infections (Hyun et al., 2018). Hassan et al. (2014) also reported that broilers fed different direct feed microbial of 0.05 % had a better dressing percentage and decreased *Escherichia coli* and *Clostridium* spp. count in the caecum. Presently, there is no information on feeding *Daniellia oliveri* extract to broilers, evaluating its efficacy to birds could exert positive effect on digestive and absorptive function in the guts as well as give a clue on the tolerable level of the extracts in broiler chicks.

Therefore, the objective of this study is to evaluate the carcass quality, nutrient retention and caeca microbial population of broiler chicks administered Rolfe (*Daniellia oliveri*) leaf extract as an antibiotic alternative.

2. MATERIALS AND METHODS

Experimental site

The experiment was carried out at the University of Abuja Teaching and Research Farm, Animal Science Section, Main Campus, along Airport Road, Gwagwalada, Abuja, Nigeria. Gwagwalada is the headquarters of the Gwagwalada Area Council and is located between latitude $8^0 57'$ and $8^0 5''$N and longitude $7^0 05'$ and $7^0 06'$E. The temperature of Gwagwalada ranges from 28-33°C in the day time and 22-25°C in the night.

Collection of test material (*Daniellia oliveri* leaf) and preparation of the extract

Fresh and healthy *Daniellia oliveri* leaves were obtained from several strands of the trees at the premises of the University of Abuja, Gwagwalada, Nigeria. The mature leaves were dark green and slightly glossy with a lighter mid veins and undersides. The plant was authenticated at the Herbarium Unit, Department of Biological Sciences, University of Abuja with a voucher specimen number DOS 121 – 2019.

Daniellia oliveri leaf was rinsed thoroughly with running tap water followed by distilled water to remove soil and other bound particles, air dried until a constant weight was obtained and made into meal using a blender.

The leaf extract was prepared by putting 250 grams of *Daniellia oliveri* meal into 1000 ml of ethanol (80% BDH) and soaked for 48hrs in an air tight container. The obtained extract (DOE) was filtered through a normal filter paper using Whatman No.1 filter paper and kept in refrigerator at 4°C for further analysis.

Experimental birds, diets and management

A total of two hundred and fifty-one-day-old broilers (Ross 308) of mixed sex were purchased from (a commercial hatchery in Ibadan) and transported to University of Abuja Teaching and Research Farm, Abuja. Prior to the commencement of the experiment, the pens were properly disinfected, and the drinkers and feeders were thoroughly washed while wood shavings were spread on the floor as litter material. The chicks were weighed individually at the beginning of the experiment; wing–banded, distributed randomly into 5

treatments of 250 chicks of five replicates each consisting of 10 birds. Vaccines were administered according to the prevailing condition in the environment. Clean feed and water were administered unrestricted and a continuous light system was used throughout the experimental period which lasted for 56 days.

Birds were fed a basal diet formulated according to NRC (1994). Starter diet (0-4 weeks) containing a crude protein (CP) of 23.23% and metabolizable energy (ME) of 2950.3 kcal/kg and finisher diet (5-8 weeks) containing 21.40 % CP and 3200.8 kcal/kg ME were given.

Experimental set-up

Treatment 1: Basal diet + 1.20 g/litre of Neomycin (Control)
Treatment 2: Basal diet + 10 ml / litre of water
Treatment 3: Basal diet + 20 ml / litre of water
Treatment 4: Basal diet + 30 ml /litre of water
Treatment 5: Basal diet + 40 ml / litre of water

Data measured and experimental design

The initial weight, final weight and feed intake were recorded weekly for each treatment. Mortality was also recorded in each group as it occurs. All other management practices were strictly adhered to. The experimental design was a completely randomized design (CRD).

Carcass quality evaluation

At the end of the 8th week, ten birds were randomly selected per treatment; they were fasted overnight, weighed, slaughtered and manually de-feathered. The carcass weight, dressed weight, weight of the visceral organ and cut parts of the birds were recorded.

Nutrient retention trial

A nutrient retention trial was carried out on the 8th week of the experiment; two birds were selected from each replicate pen making a total of ten (10) birds per treatment. The birds were housed in cages with wire bottoms. Trays were placed under each cage for fecal collection. The birds were given a known amount of feed for seven days and clean water was also given throughout the experiment. Feed consumed was measured by weighing the left over feed daily and subtracting from amount of feed provided. Excreta was collected for 7days, dried and mixed thoroughly. Contaminants were carefully removed and the excreta were stored in containers. Samples were subsequently oven dried at 80^{o}C and taken for proximate composition in the laboratory using the methods described by Association of Analytical Chemist (AOAC, 2000). The percentage retention was calculated using the equation below:

$$\text{Nutrient retention} = \frac{\text{Nutrient intake (DM)} - \text{Nutrient output (DM) in the excreta}}{\text{Nutrient intake (DM)}} \times 100$$

Caeca microbial population

At the end of the experiment (56 days), caeca microbial count was conducted using 5 birds per treatments (the same birds used for carcass evaluation). A 10-fold serial dilution method, in which of 1% peptone solution was mixed with caeca samples and poured on Mac Conkey agar plates and *lactobacilli* medium III agar plates, was used to determine the colony forming unit (cfu) in each gram of caeca sample by means of pour plate method. *E. coli* was cultured on Eosin Methylene Blue (EMB) agar at 37℃ for 48 h. *Lactobacilli* was enumerated on Rogosa, Sharpe agar at 37℃ for 72 hours. Colonies with metallic green sheen colours were considered as *E. coli* while the white colour colonies were identified as lactobacilli. *Salmonella typhi*, *Salmonella aeruginosa* and *Pseudomonas* were enumerated on Rins, Sharpe agar at 37℃ for 72 hours and their count was enumerated on metallic sheets as brown, grey and purple respectively.

Laboratory analysis

Vitamin content (ascorbic acid, riboflavin, niacin and β-carotene) of *Daniellia oliveri* leaf was analyzed using the methods reported by Onwuka (2005), while amino acids were analyzed using commercial diagonistic kits (Humburg, Braunschweig, Germany, Model- 3401-UI-OF45).

Statistical analysis

All data were subjected to one -way analysis of variance (ANOVA) using SPSS (23.0) and significant means were separated using Duncan multiple range tests (Duncan, 1955). Significant was declared if $P \leq 0.05$.

Table 1 Ingredient composition of the experimental diets

Ingredients	Starters mash (0-4 weeks)	Finishers mash (5-8 weeks)
Maize	52.00	60.00
Wheat offal	2.50	5.00
Soya bean meal	30.00	25.00
Groundnut cake	8.00	4.00
Fish meal (72%)	2.00	2.00
Limestone	1.50	1.50
Bone meal	3.00	3.00
Lysine	0.20	0.20
Methionine	0.20	0.20
*Premix	0.25	0.25
Salt	0.30	0.30
Toxin binder	0.10	0.10
Calculated analysis (% DM)		
Crude protein	23.10	21.40
Crude fibre	4.18	5.01
Ether extract	4.03	4.47
Calcium	1.50	1.60
Phosphorus	0.58	0.66
Energy (Kcal/kg)	2910.3	3200.8

***** Premix supplied per kg diet: - vit A, 13,000 I.U; vit E, 5mg; vit D3, 3000I.U, vit K, 3mg; vit B2, 5.5mg; Niacin, 25mg; vit B12, 16mg; choline chloride, 120mg; Mn, 5.2mg; Zn, 25mg; Cu, 2.6g; folic acid, 2mg; Fe, 5g; pantothenic acid, 10mg; biotin, 30.5g; antioxidant, 56mg

Table 2 Vitamin composition of *Daniellia oliveri* leaf meal

Vitamin	Composition (mg/ 100 g)
Ascorbic acid	34.09
β –carotene	811.3
Niacin	1.16
Thiamine	1.21
Riboflavin	1.02
Folic acid	2.77

Table 3 Amino acid composition of *Daniellia oliveri* leaf meal

Amino acid	***D. oliveri* (%)**
Essential amino acid	
Threonine	1.89
Leucine	5.11
Lysine	2.88
Valine	5.93
Tryptophan	1.03
Glycine	4.33
Phenyl alanine	5.61

Histidine	6.33
Methionine	0.77
Non-essential amino acid	
Alanine	5.89
Serine	4.09
Proline	6.08
Aspartic acid	7.11
Glutamic acid	10.22
Tyrosine	1.04
Cysteine	1.00

Table 4 Effect of treatments on organ weights and primal cut parts of broiler chicks

Parameters (g)	T1	T2	T3	T4	T5	SEM
Final live weight	1847.1^{c}	1996.5^{b}	1980.3^{b}	2102.2^{a}	2139.3^{a}	14.95
Dressed weight	1197.0^{c}	1346.5^{b}	1330.3^{b}	1452.2^{a}	1489.3^{a}	32.30
Dressing %	65.00^{c}	67.44^{b}	67.18^{b}	69.08^{a}	70.00^{a}	0.07
Breast muscle (%)	18.78^{b}	21.60 ^{a}	22.86^{a}	22.93^{a}	22.97^{a}	0.94
Shank (%)	10.77^{a}	10.14^{a}	10.65^{a}	10.77^{a}	11.72^{a}	0.21
Thigh (%)	8.03^{c}	8.07^{b}	9.12^{a}	9.29^{a}	10.06^{a}	0.41
Wings (%)	7.82^{c}	8.91^{b}	9.72^{a}	9.85^{a}	9.64^{a}	0.38
Drum stick (%)	9.95^{a}	10.41^{a}	10.48^{a}	10.57^{a}	10.93^{a}	0.55
Head (%)	2.21^{b}	2.28^{b}	2.23^{b}	2.64^{a}	2.88^{a}	0.10
Neck (%)	3.69^{a}	3.77^{a}	4.11^{a}	4.27^{a}	4.20^{a}	0.22
Back (%)	16.77^{a}	17.22^{a}	17.13^{a}	18.77^{a}	18.22^{a}	1.51
Organs						
Heart (%)	0.55	0.58	0.60	0.58	0.54	0.01
Liver (%)	2.63^{b}	2.07^{c}	2.06^{c}	2.48^{c}	3.26^{a}	0.24
Gizzard (%)	2.02^{b}	2.21^{b}	2.67^{a}	2.38^{a}	2.19^{b}	0.03
Spleen (%)	0.10^{a}	0.13^{a}	0.12^{a}	0.15^{a}	0.14^{a}	0.03
Pancreas (%)	0.30^{c}	0.38^{b}	0.44^{a}	0.49^{a}	0.49^{a}	0.01
Kidney (%)	1.03^{b}	1.20^{b}	0.99^{b}	1.29^{b}	2.66^{a}	0.28
Intestine (cm)	110.3^{c}	118.0^{c}	133.2^{b}	141.0^{a}	147.3^{a}	4.73

Means in the same row with different superscript are significantly different (P<0.05)

Table 5 Effect of treatments on nutrient retention of broiler chicks

Parameter (%)	T1	T2	T3	T4	T5	SEM
Dry matter	71.00^{b}	72.11^{c}	74.61^{b}	83.18^{a}	85.64^{a}	2.31
Crude protein	74.56^{a}	71.04^{b}	75.45^{b}	77.12^{a}	79.14^{a}	2.01
Ether extract	61.27^{a}	57.23^{b}	60.67^{a}	61.56^{a}	66.34^{a}	3.51
Crude fibre	39.26^{c}	43.91^{b}	42.10^{b}	44.13^{b}	48.22^{a}	6.60
NFE	67.22^{b}	56.11^{c}	65.12^{c}	78.37^{a}	80.34^{a}	2.98

Means in the same row with same superscripts are significantly different (P<0.05)

NFE, nitrogen free extract; SEM, standard error of mean

Table 6. Effect of dietary treatments on the caeca microbial population of broiler chicks

Treatments (cfu/g)	*E.coli*	*Lactobacillus*	*S. typhi*	*P.aeruginosa*	*S. aureus*
T1	25.08^{a}	15.54^{e}	26.92^{a}	31.36^{a}	16.54^{a}
T2	17.96^{b}	24.44^{d}	21.66^{b}	27.78^{b}	12.52^{b}
T3	14.15^{c}	32.08^{c}	16.90^{c}	26.00^{c}	10.42^{c}
T4	11.67^{d}	39.14^{b}	12.30^{c}	24.92^{c}	10.32^{c}
T5	9.000^{e}	47.00^{a}	10.78^{d}	23.10^{d}	9.85^{c}
SEM	0.34	0.48	0.30	0.24	0.20

Means with different superscripts in the same row are significantly different (P<0.05)

3. RESULTS

Vitamin analysis of *Daniellia oliveri* leaf meal

Table 2 showed the vitamin composition of *Daniellia oliveri* leaf meal. The results revealed *Daniellia oliveri* leaf meal as a good source of ascorbic acid (39.09 mg/100 g), folic acid (2.77 mg/100 g), β –carotene (811.3 mg/100 g), thiamine (1.21 mg/100 g), niacin (1.16 mg/100 g) and riboflavin (1.02 mg/100 g).

Amino acid analysis of *Daniella oliveri* leaf meal

The amino acid composition of *Daniellia oliveri* leaf meal is presented in Table 3. Results revealed the presence of threonine, leucine, lysine, valine, tryptophan, glycine, phenylalanine, histidine, methionine, alanine, serine, proline, aspartic acid, glutamic acid, tyrosine and cysteine at 1.89, 5.11, 2.88, 5.93, 1.03, 4.33, 5.61, 6.33, 0.77, 5.89, 4.09, 6.08, 7.11, 10.22, 1.04 and 1.00 (%) respectively.

Carcass characteristics of broiler chicks administered *Daniellia oliveri* leaf extract

The carcass characteristics of the experimental chicks shown in Table 4. Final live weight, dressed weight (DW) and dressing percentage (DP), which ranged between (2139 – 1847.1 g), (1489.3 – 1197.0 g) and (70.0 – 65.0 %), were lowest in T1 and highest in T4 and T5. Liver (expressed as % of DW) (3.26 – 2.06 %) was higher in T5 than in T2, T3 and T4 which in turn were higher than T1 (P<0.05). Wings (9.85 – 7.82 %), pancreas (0.49 – 0.30 %) and thigh (10.06 – 8.03 %) were lower (P<0.05) for T2 relative to other treatments which were similar (P>0.05). Gizzard was (P<0.05) higher for T3, T4 and T5 compared with T1 and T2. Intestine length was longest for T4 and T5 and shortest for T1 and T2 (P<0.05). Heart was similar (P>0.05) among the treatments.

Nutrient retention of broiler chicks administered *Daniellia oliveri* leaf extract

Table 5 reveals the effect of treatments on nutrient retention of broiler chicks. Dry matter retention was highest for T4 (83.18 %) and T5 (85.64 %) and lowest for T1 (71.00 %) (P<0.05). CP retention was higher (P<0.05) for T3 (75.45 %), T4 (77.12 %) and T5 (79.14 %) than for T1 (74.56 %) and T2 (71.04 %). Fat retention was lower (P<0.05) for T2 than for other treatments. CF retention was highest (P<0.05) for T2, T3, T4 and T5 and lowest for T1 (39.26 %). NFE retention was highest (P<0.05) for T4 (78.37 %) and T5 (80.34 %) and lowest for T2 (56.11 %) and T3 (65.12 %) (P<0.05).

Effect of *Daniellia oliveri* leaf extract on caecal microbial population of broiler chicks

The effect of treatments on the logarithmic count of microbial population in caeca of broiler chicks is presented in Table 6. *E. coli* count ranged between 9.00 and 25.08 (cfu/g), Lactobacillus count 15.54 and 47.00 (cfu/g), *Salmonella typhi*10.78 and 26.92 (cfu/g), *Pseudomonas aeruginosa* 23.10 and 31.36 (cfu/g) and *Salmonella aureus* 9.85 and 16.54 (cfu/g). Except for Lactobacillus, which higher (P<0.05) in the Daniellia leaf extract treatments, the leaf extract treatments reduced (P<0.05) *E. coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Salmonella aureus* counts in the caecum when compared to the control treatment.

4. DISCUSSION

The leaves of *Daniellia oliveri* possess significant quantities of water soluble vitamins – ascorbic acid and vitamin A precursor, β –carotene, folic acid, niacin, thiamine and riboflavin. According to Bakare *et al.* (2010) vitamins are a diverse group of inorganic molecules required in small quantities in the diet for health, growth and survival. Vitamin A is an important component which aids clearer sight (Bakare *et al.*, 2010). Vitamin C helps maintains blood vessel flexibility circulation and acts as anti-stress (Omale *et al.*, 2009). Riboflavin (Vitamin B_2) is synthesized by all green plants, most bacteria, yeast and moulds. Animals have so far not been shown to synthesize

riboflavin. Persons deficient in vitamin B_2 show keratitis, coerneal vascularization, glossitis, cheilosis and seborrheic dermatitis (Jain et al., 2005). Since the *Daniellia oliveri* leaf contains reasonable amounts of vitamins, it could a good source of vitamins in animal nutrition.

The dressed weight usually expressed as dressing percentage is an indication of meat value that could be obtained from an animal. The higher dressed weight, dressing percentage, prime cuts and organs weights of the birds given DOE than the birds given conventional neomycin antibiotic resulted from the higher live weight of the DOE treated birds, in consistence with the previous reports (Obun *et al*., 2012). Similar findings were obtained by Barreto *et al.* (2008) who used garlic oil as a natural feed additive for Hubbard broiler chicks and reported improved carcass quality of the birds. Alabi *et al.* (2016) observed that inclusion of aqueous *Moringa olifera* leaf extracts at amounts up to 150 ml/liter improved the carcass quality and performance of birds. The present findings, however, disagree with the findings of Togun *et al.* (2006) who noted that live and carcass weight of broiler chicks were reduced by wild sunflower forage meal inclusion above 10% level in the ration. Ochi et al. (2015) noted non-significantly affected carcass characteristics in broilers supplemented with *Moringa olifera* seed powder at 2%. The smaller organ weights, particularly of liver and kidney, indicate the non-toxicity of the secondary metabolite concentrations of DOE. Bamgbose *et al.* (2004) reported that dressed weight and organ weight characteristics are veritable signs or indicators of the level of reduction or otherwise of anti-nutritional factors. There was no enlargement or atrophy of the internal organs beyond normal thus indicating that the birds were able to tolerate *Daniellia oliveri* leaf extract.

The improved nutrient retention of birds on DOE treatment is in agreement with previous research (Hernandez et al., 2004) who reported that plant extract supplementation improved apparent whole tract digestibility of nutrients. The improvement of nutrient retention of broiler chicks given different levels of DOE was probably due to herbal effects in increasing the microbial population in the intestine. The efficacy of any dietary feed additives observed under less hygienic housing conditions, especially under the separate floor pens equipped with wood shavings as litter stimulates the activities of the feed additive. The isoprene derivatives, flavonoids and other plant metabolites may affect the physiological and chemical function of the digestive tract. The stabilizing effect on the intestinal microflora may be associated with immediate nutrient metabolism (Jamrozand Karmel, 2002).

The inclusion of DOE across the treatments increased the beneficial *Lactobacillus* spp in T5 compared with T1. It is well documented that herbs and phytogenic extracts suppress harmful microorganisms and stimulate beneficial microbes such as *Lactobacillus spp* (Hassan et al., 2014). *Lactobacillus spp* is a normal member of intestinal microflora of animals with a positive impact on regular gut function (Ahmad, 2006). Therefore, increasing the number of *Lactobacillus spp* could play a role in preventing the mortality of the birds on the treatment diets. *A lactobacilli bacterium also activates* the intestinal immune system and increase the resistance to diseases through the releasing of low-molecular weight peptides which induce immune activation (Simon et al., 2001).

DOE also decreased *Escherichia coli* and other pathogens compared to the control. Hanan (2015) observed a reduced bacterial diversity in the caecum of birds fed a diet supplemented with 4 % turmeric (*Curcuma longa*). A reduction in the number of pathogenic bacteria changes the microbial ecology in favour of beneficial species in the intestine (Lee et al., 2010) resulting in improved ability of epithelial cells to regenerate villus and thus enhances intestinal absorptive capacity. Such effects reveal the antimicrobial activity of additives mixture to control pathogenic bacteria. Bioactive compounds in DOE display antimicrobial action against *Escherichiacoli* and many other pathogens. According to Carson et al. (2002), secondary metabolites in plants exhibit wide range of antibacterial activities against Gram-positive and Gram-negative bacteria. Phenolic compounds in DOE also have the ability to cause structural and functional damage to cytoplasm membranes of harmful bacteria (Lambert et al., 2001).

5. CONCLUSION

It can be concluded from the experiment that *Daniellia oliveri* extract could be given to broiler chicks up to 40 ml/litre without any deleterious effect on the performance and carcass characteristics of the animals. Reduction of *Escherichia coli*, other pathogenic organisms with an increase of *Lactobacillus* in the caeca of birds orally administered with *Daniellia oliveri* extract could be commercially interesting as it may have the potential to be an alternative to antibiotic growth promoter for broiler chickens.

Funding:
This study has not received any external funding.

Conflict of Interest:
The authors declare that there are no conflicts of interests.

Peer-review:
External peer-review was done through double-blind method.

Data and materials availability:

All data associated with this study are present in the paper.

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